CHAPTER 1

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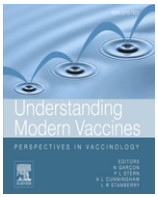
Understanding Modern Vaccines:
Perspectives in Vaccinology



Vaccine evolution

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Key concepts

- Vaccines have made the second most major contribution to the control and eradication of infectious diseases after the distribution of clean water
- Modern vaccine concepts stem from early empirical approaches to variolation and vaccination
- The germ theory opened the door to a more relevant knowledge-based vaccine development process
- Since the late 18th century, several important techniques to produce effective vaccines have been developed:
  - Attenuation and inactivation of pathogens at end of the 19th century
  - Toxoids and bacterial cancer immunotherapy in the 1920s
  - Use of adjuvants in the 1920s
  - Embryonated eggs to grow viruses in the 1930s
  - Cell cultures to grow viruses in the 1950s
  - Vaccines based on split pathogens or subunits in the 1970s
  - Recombinant DNA approach in the 1980s
  - Conjugation of polysaccharides to protein carriers in the 1980s
  - Reassortment of viral genes in the 1990s
  - Dendritic cell vaccines for cancer treatment in 2010

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Vaccines have dramatically improved human health. The steady decline in deaths in children under 5 years of age is attributable to the increasing availability of vaccines in the developing world.

A growing knowledge of immunology has increasingly influenced vaccine design in the past century, leading to the production of new types of vaccines (whole cell, live or inactivated, subunit, recombinant proteins etc) with associated advantages and challenges. In addition, public health priorities have evolved over time. The first vaccines were developed against diseases with high morbidity and mortality rates, such as smallpox, diphtheria and tetanus. In addition, 'battlefield diseases' – particularly in the era of trench warfare – including typhoid fever, plague and cholera, drove the development of early vaccines.

More recently, the drivers for vaccine development have changed, reflecting changes in global society. Although highly pathogenic infectious diseases remain the principal targets for effective *vaccination*, assessments of benefit versus risk and consideration of health economics are now an obligatory part of the development process. Better understanding of immunology and pathogenesis of the targeted diseases facilitates identification of the type and quality of immune responses that are desirable for each new prophylactic or *therapeutic vaccine*.

Smallpox and polio – two success stories

In December 1979, the World Health Organization (WHO) announced the eradication of smallpox following successful vaccination campaigns throughout the previous two centuries. Another disease presently close to eradication is polio (Figure 1.1). The WHO declared that the Americas were polio-free in 1994, followed by the Western Pacific region in 2000 and the European region in 2002. In the past 20 years, polio cases have decreased from an estimated 350,000 annual cases to 1640 cases in 23 countries in 2009, the majority of which (69%) occurred in Nigeria and India.

Figure 1.1 Hospitalised victims during the polio outbreak of the 1950s. During the polio epidemics of the 1950s, entire wards were filled with people obliged to rely on an 'iron lung' due to paralysis of the respiratory muscles. Some patients would remain this way for the rest of their lives.

March of Dimes Foundation.

Vaccination – how and where did it start?

In the ancient world, it was common knowledge that a person was rarely infected twice with the same disease and the term 'immunity' was first used in reference to plague in the 14th century.

Figure 1.2 Child with smallpox. The last naturally occurring case of smallpox was reported in 1977 in Somalia.

Centers for Disease Control and Prevention.

Inoculation is a long-established practice that protected humans and animals from contagious diseases – it has been practised in various Asian and African countries for centuries. Madhava Nidana, a classical text of traditional Ayurveda, is one of the first written reports of attempts to inoculate and dates back to 7th century India.

The development of natural sciences and experimental methods during the 18th century led to the systematic use of inoculation to fight one of the most significant threats of this era, smallpox, also known as the 'speckled monster' (Figure 1.2).

VARIOLATION AND INOCULATION

Inoculation, or *variolation* in the case of smallpox, involved subcutaneous administration of liquid taken from a pustule of a person showing mild clinical symptoms, and represented the precursor to live pathogen vaccines. In Europe, the new methods of variolation quickly became known amongst physicians. Since there was an increasing demand for protection against smallpox, physicians soon began the variolation procedure on a large scale. However, variolation was not without its attendant risks; there were concerns that recipients might spread smallpox to others, or develop a systemic infection. Approximately 2–3% of variolated persons died from the disease, or suffered from other diseases such as tuberculosis (TB) or syphilis transmitted by the human to human inoculation procedure. Despite the risks, mortality associated with variolation was 10 times lower than that associated with naturally occurring smallpox. During a smallpox epidemic in Boston in 1721, half of the 12,000 population was infected and mortality was 14%; in comparison, mortality in variolated individuals was only 2% (Blake, 1959).

VACCINATION

The use of cowpox as a vaccine for smallpox is generally seen as a remarkable advance over variolation. Variolation used human material, including *serous* matter from pustules and scabs taken

Figure 1.3 Lady Montague. In the early 18th century, variolation was introduced to England by Lady Mary Wortley Montague, following her experience of the procedure in Turkey.

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from a patient with a mild case of the disease, and generally conferred strong, long-lasting immunity. The first smallpox vaccine for general use was introduced by Edward Jenner in 1796 (there

was a private inoculation of his family by a farmer named Jesty in 1774 prior to Jenner's inoculation) based on anecdotal observations that milkmaids infected by cowpox, a benign infection for humans, were subsequently immune to smallpox. By deliberately inoculating people with small doses of cowpox from pustules on the udders of infected cattle, Jenner demonstrated that protection against smallpox could be achieved (Figure 1.4). The first person he inoculated was James Phipps on the 14 May 1796; he later challenged him with fresh smallpox pustular material. Through a form of cross-protective immunity, cowpox vaccination provided humans with satisfactory protection, although it was probably less durable than that produced by inoculation with smallpox. Jenner called this preventive measure 'vaccination' (vaccinia, from Latin *vacca* = cow) and his practice of inoculation against smallpox using cowpox became widely accepted by the end of the 18th century. The Royal Jennerian Society was founded in London in 1803, and in a period of 18 months approximately 12,000 individuals were vaccinated.

Cowpox vaccination was made more efficient by performing human arm-to-arm transmission of infectious cowpox fluid, which greatly increased the capacity for providing vaccinations to larger numbers of people as it did not rely on the sporadic outbreaks of cowpox in cattle. However, this method was not without problems, including an apparent decline in the potency of the vaccine which necessitated revaccination in order to maintain immunity and the concomitant transmission of other infections.

During the latter half of the 19th century, cows and calves were again used as a lymphatic fluid source to re-obtain a potent cowpox-based vaccine. Following the observation that the quality of the isolated fluid rapidly declined, Robert Koch recommended that glycerine be added to kill contaminating bacteria. This preservation method soon became standard practice.

Many inoculation techniques were used for smallpox vaccination over the years. When improvements in vaccine potency resulted

Introduction of variolation in Europe and North America

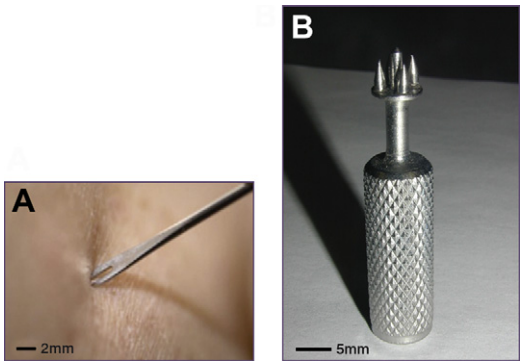
Lady Montague (Figure 1.3), who had survived infection with smallpox (variola) herself, was so impressed with the method of variolation used in the Ottoman court (which involved cutaneous inoculation of smallpox pus) that she ordered the embassy surgeon, Charles Maitland, to inoculate her 5-year-old son. Upon their return to London in 1721, Lady Montague instructed Maitland to inoculate her 4-year-old daughter in the presence of physicians of the royal court. The results convinced the Princess of Wales to inoculate her own children in the same way. As a result, the procedure was generally accepted and became quite popular. Simultaneously, variolation was also first practised in 1721 in Boston using knowledge gained from an African slave, Onesimus, who was inoculated as a child in Africa.

Figure 1.4 Smallpox inoculation procedure in the 18th century. In 1796, Edward Jenner, a general practitioner and surgeon, inoculated 8-year-old James Phipps with material from cowpox blisters of a milkmaid. The boy developed a mild fever and was subsequently immune to smallpox.

Collection of the University of Michigan Health System, gift of Pfizer Inc. UMHS.23.

in excessively severe reactions with the inoculation techniques practised so far, multiple puncture methods, eg using a bifurcated (two-pronged) needle (Figure 1.5, panel A) or scarification

Figure 1.5 Multiple puncture needles used for smallpox inoculation. Needles used to prick the skin during inoculation with smallpox. A: bifurcated needle; B: scarification instrument.



Centers for Disease Control and Prevention (A). Courtesy of Professor Myron Levin (B).

The first vaccination programme in history

The New World was ravaged by smallpox for several centuries after the Spanish conquest. In 1804, 6 years after Jenner's publication, the first and little known effort to eradicate smallpox for good was commissioned by Charles IV of Spain, in response to a large outbreak of smallpox in the Spanish colonies. Known as the Royal Philanthropic Expedition, King Charles IV appointed Francisco Xavier de Balmis to take Jenner's vaccine to the Spanish colonies, the Philippines and China. During the journey from Spain to the New World, the vaccine was kept viable by passing it from arm-to-arm in orphaned children, who were brought along expressly for that purpose.

instrument (Figure 1.5, panel B), were implemented. However, the simple cut or scratch technique also remained popular throughout the smallpox vaccination period.

THE GERM THEORY OF DISEASE

Until the late 19th century, diseases were commonly believed to be caused by an invisible agent, a miasma, and were 'spontaneously generated' in response to 'bad air' and other environmental triggers. Infectious illnesses were also believed to be caused by imbalances in the body. While Jenner had no knowledge of microorganisms and viruses, progress in microbiology from the late 19th century onwards developed into the modern concept of communicable diseases. Hence, further advances in *vaccinology* were gained from an understanding of what caused infectious diseases — the science of aetiology and host–pathogen interactions.

Through the pioneering research of Louis Pasteur and Robert Koch, who established that microorganisms were the cause of infectious diseases, the science of immunology was founded. Pasteur disproved the spontaneous generation theory of *microbes*, and his studies of the metabolism of microorganisms led to the discovery of ways in which microbes could be transformed so as to produce vaccines and other new ways to prevent and treat infection. Koch demonstrated that infectious agents transmit diseases and his four postulates established a specific agent as the cause of a disease. Today, Koch's postulates (Table 1.1) are still sound principles for determining causality. An overview of discovery of some specific pathogens and the availability of vaccines for diseases caused by these pathogens is shown in Figure 1.6. It can be seen from this figure that in the case of smallpox, a successful vaccine could be developed without knowledge of the actual nature of the causative agent.

ATTENUATED AND INACTIVATED PATHOGENS

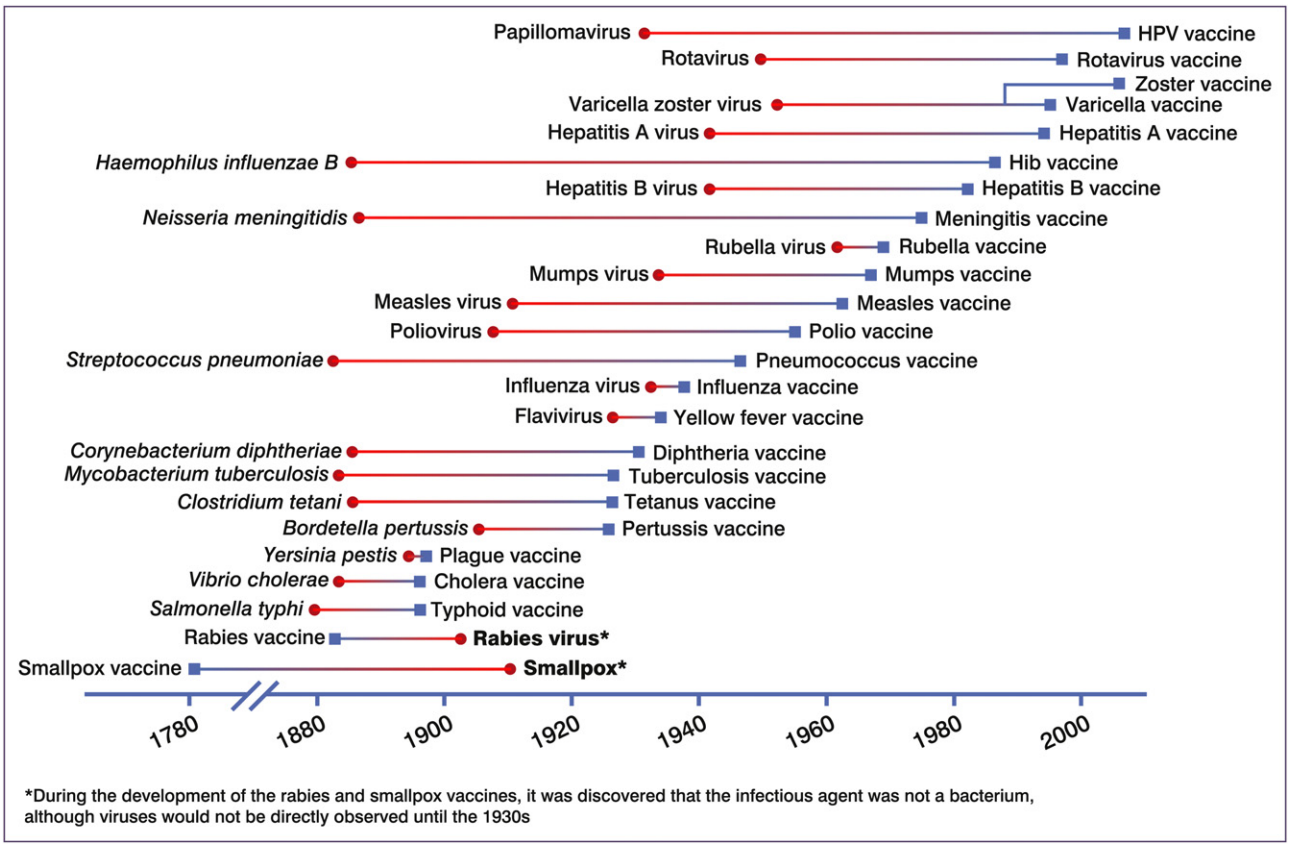
Pathogen attenuation was used to develop vaccines in Pasteur's laboratory by Émile Roux in the late 1870s, when he suspended

TABLE 1.1. KOCH'S POSTULATES

1. The microorganism must be identified in any stage of disease in the infected host.
2. The microorganism must be isolated from the host and grown in pure culture outside the host.
3. Infection of healthy susceptible hosts with the cultured microorganism must result in the same symptoms of the disease.
4. The microorganism must be re-isolated from the experimentally infected host and shown to be identical with the original isolate.

Adapted from Koch (1884).

Figure 1.6 Pathogen isolation and vaccines. Depending on availability of appropriate technology, there may be considerable variations in time between pathogen identification (●) and development of a vaccine (■). In the case of smallpox, a vaccine was available long before viruses as causing agents were known. The rabies vaccine was also developed before knowing the causative agent. A pathogen, like varicella zoster virus, may cause different diseases (varicella and zoster) for which separate vaccines have been developed.
HPV, human papillomavirus; Hib, Haemophilus influenzae type b.



How attenuation of pathogens was discovered

Pasteur developed methods for the attenuation of pathogens thanks to the involuntary negligence of one of his co-workers in his laboratory, who left an avian cholera culture (*Pasteurella multocida*) exposed to air for an extended period of time prior to inoculation experiments in chickens. This resulted in a revolutionary discovery, as the cultured microbes lost their ability to induce disease in chickens, but left these chickens immune to a virulent culture of avian cholera. Pasteur concluded that weakened microbes could provide, in general, immunity to infectious diseases.

Attenuation of vaccines

An attenuated vaccine contains an infectious, but less virulent, pathogen that induces a mild form of disease. Attenuated vaccines typically stimulate strong, durable antibody- and cell-mediated immune responses. An attenuated vaccine has the disadvantage of potentially being associated with a small risk of vaccine-related disease, especially in individuals with underlying impairment of immune function. Furthermore, for some attenuated vaccines, there are safety concerns about the potential for reversion from the attenuated form back to a virulent one.

the spinal cord of a rabbit infected with rabies in a flask in a warm dry atmosphere to achieve slow desiccation of the infectious material. This produced a weakened substance for inoculation.

This practice rendered the microorganisms less pathogenic but still *immunogenic*. Pasteur and his team then succeeded in producing attenuated microorganisms of different strengths by varying the desiccation time. On 6 July 1885, a 9-year-old boy, Joseph Meister, became the first human to be successfully vaccinated with a *live, attenuated vaccine* against rabies. This method was not widely accepted however, as a few fatal cases of rabies in those vaccinated were attributed to the vaccine and viewed as 'medical murders', in spite of the fact that hundreds of people exposed to rabies had been saved from death as a result of vaccination.

Almost in parallel to attenuated pathogens, researchers started working on inactivated pathogens. These were initially developed for veterinary applications, based on the observation that inactivated pathogens maintained the ability to induce protection. The first *inactivated vaccines* developed for human use were against typhoid, cholera and plague.

There are, however, some disadvantages associated with inactivated whole pathogen vaccines. Multiple doses are generally needed to provide sufficient stimulation of the immune system and *booster* doses may be needed to induce or maintain persistent immune responses. While live, attenuated and inactivated pathogen vaccines were effective, in the early days of vaccine manufacturing there were many issues including contamination, potency and quality of pathogen production, and lack of standardised harvesting processes.

At the end of the 19th century, many of the fundamental aspects of vaccinology were in place because of the pioneering work of scientists like Pasteur, Koch, Metchnikoff and Ehrlich. The most important advance was the demonstration that the administration of pathogens, either attenuated or inactivated, resulted in

Figure 1.7 Typhoid Mary. Mary Mallon (1869–1938), nicknamed 'Typhoid Mary', gained notoriety as history's most famous chronic carrier of an infection. She was a cook employed in various homes and institutions, and was known to have been the cause of 51 cases of typhoid fever, even though she was apparently healthy. She was confined to a New York hospital for the last 24 years of her life.

Image — believed to be public domain.

protection against the disease caused by the respective native pathogen. Developments in pathogen attenuation processes led to consistent production of attenuated microbes, and many of the vaccines employed today are still based on these developments. Figure 1.8 shows the various vaccine technologies developed over time.

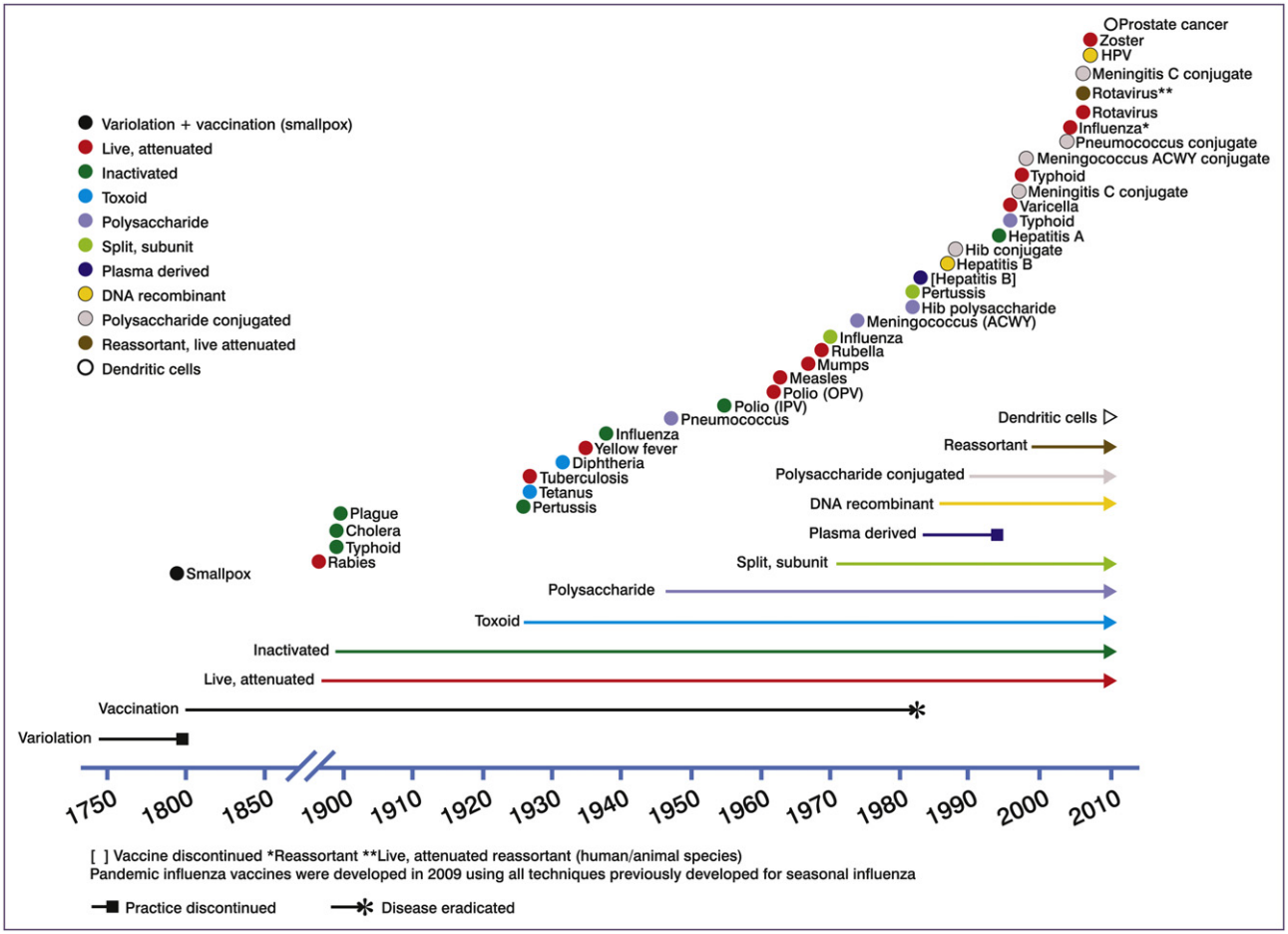
How inactivated vaccines were discovered

Formaldehyde was used in Gaston Ramon's laboratory to clean and sterilise test tubes and glass flasks. One of the flasks used for toxin preparation was not thoroughly rinsed and the remaining formaldehyde was sufficient to inactivate bacterial toxins (1924). This observation appears to have originated the use of formaldehyde inactivation in vaccines.

Typhoid fever vaccine and the army

Typhoid fever, a disease spread easily under primitive sanitary conditions and by chronic carriers (Figure 1.7), was highly feared at the beginning of the 19th century due to its high case-fatality rate of up to 20%. To protect troops against typhoid fever, the military initiated the development of a whole cell, inactivated bacterial vaccine. Typhoid vaccination was first tested in 1896 in 2835 volunteers of the Indian army (Levine, 2008). Consequently, the army decided to vaccinate soldiers sent to the Boer War. The vaccine caused some adverse events but a committee reviewed the available data and concluded that the benefits from prevention of the disease outweighed the risks from vaccination; this may be the first example of an assessment of the risks and benefits of vaccination.

Figure 1.8 Vaccines and technologies. Vaccine development timeline from the first practice of variolation. Most of the technologies are still used for the development of vaccines. Plasma-derived vaccines have not been used in most countries since the 1990s.
IPV, inactivated polio vaccine; OPV, oral polio vaccine; HPV, human papillomavirus.



TOXINS, TOXOIDS AND IMMUNOTHERAPY

At the end of the 19th century, Émile Roux and Alexandre Yersin discovered that diphtheria and tetanus bacilli produce soluble molecules called *exotoxins*, which caused the symptoms of these infections. Soon after this discovery, Emil von Behring and

Shibasaburo Kitasato postulated the serum *antitoxin* concept. The use of the term 'immunisation' dates from this work, referring to the rabbit serum that contained the antitoxin as immune serum.

In 1924, Gaston Ramon, a veterinarian at the Pasteur Institute, applied chemical inactivation to bacterial toxins to produce *toxoids* of diphtheria and tetanus. By this method, he transformed the tetanus toxin with formaldehyde and heat into a safer, non-toxic product, without changing its immunogenic potential. He called this chemically treated product 'anatoxin' (ie toxoid). This discovery was also applicable to the toxin produced by the diphtheria bacillus. The diphtheria toxoid produced by this method was used in a vaccination programme to greatly minimise fatal cases of diphtheria in infants. The tetanus toxoid vaccine was widely used to prevent tetanus from battle wounds sustained during World War II. The introduction of tetanus vaccination has almost eliminated the number of cases in developed countries; however, tetanus remains a problem, largely in the developing world (Figure 1.9). Worldwide annual deaths in 2004 from tetanus were estimated to be 163,000, 144,000 of which occured in children less than 5 years of age (WHO, 2009).

Another major contribution to the development of vaccines in the early 1920s came with the discovery that certain substances could increase yields of immune sera containing antitoxins. The research carried out by Alexander Glenny and Gaston Ramon was primarily intended to increase the yields of hyperimmune sera produced in animals such as horses, rather than to increase the active immune response after antigenic stimulation in humans. Ramon noted that horses that spontaneously developed abscesses at the injection site produced greater serum antitoxin *titres*. Subsequently, he successfully described a group of substances, ranging from starch, bread crumbs and tapioca (used because they contained starch), which, when injected in conjunction with toxoids, enhanced the immune response. These substances were tested because it was already known that they caused aseptic abscesses/inflammation. The term *adjuvants*, from the Latin *adjuvare* ('to help or to aid'), was coined by Ramon to describe these substances. Around the same

Figure 1.9 Tetanus case. Neonatal tetanus is still a risk in the developing world.

Centers for Disease Control and Prevention.

Key principles of the immune response were elucidated in the late 19th century

Host cells that ingest and destroy invading microbes were identified by Élie Metchnikoff and named 'phagocytes' (literally 'eating cells' from the Greek). Understanding the way in which hosts and pathogens interact began to unravel some of the mysteries of infection and disease. This led to the concept of 'natural immunity' to infection, which was indispensable for vaccine design. In 1908, Metchnikoff was awarded the Nobel Prize in Medicine jointly with Paul Ehrlich for their work on the theory of immunity.

First Nobel Prize in Medicine

The discovery of antibodies in 1890 and passive immunotherapy of diphtheria was honoured in 1901 when the first Nobel Prize in Medicine was awarded to Emil von Behring.

The burden of diphtheria before the availability of vaccines

From 1920 to 1940, between 40,000 and 70,000 cases of diphtheria were recorded each year in the UK, but by the 1990s the number was around 10 cases a year.

time, aluminium salts were tested by Glenny (1926) to increase the immune response to the diphtheria toxoid in horses and, shortly after, in humans. Today, 80 years later, aluminium salts still remain the most commonly used adjuvant in vaccines (see *Chapter 4 — Vaccine adjuvants*).

EMBRYONATED EGGS

In 1931, Ernest Goodpasture introduced the use of embryonated hen's eggs as a medium for growing viruses. This technique represented a major advance since, until its introduction, human viruses could only be grown in animals such as ferrets and mice. The chick embryo proved to be a cheaper and safer medium for the culture of viruses. Using the egg system, Max Theiler at The Rockefeller Foundation developed an effective vaccine for yellow fever in the 1930s and received the Nobel Prize in Medicine in 1951. Vaccines against typhus that were important for troops in World War II were also developed during the 1930s. The first influenza vaccine was developed in 1940, and was a live, attenuated virus produced in hen's eggs. However, due to the instability of the viral genome, the viruses mutated rapidly and were not attenuated consistently, causing outbreaks of disease in vaccinees, which led to the discontinuation of the product for safety reasons.

THE CELL CULTURE ERA

In 1906, Albert Calmette and Albert Guérin started to culture *Mycobacterium bovis* bacillus in a potato medium to which glycerin and beef bile were added to strip the lipids from the waxy capsule of the microorganism. After 13 years and 230 passages through the medium, they obtained an attenuated strain, bacillus Calmette–Guérin (BCG). The first BCG vaccine became available in 1927 and is still widely used for the prevention of disseminated TB and TB meningitis in children.

During the 1930s, the use of animal cell cultures to grow pathogens became available. This important new technology for

the development of vaccines replaced the practice of deliberate animal-to-animal transmission of infection.

The first virus grown in a chicken kidney cell culture was the vaccinia (cowpox) virus. Following these first experiences with animal cell cultures, viruses were cultivated in human cells, either directly in primary cell cultures or in immortalised (continuously growing) cell lines. Vaccine development shifted into a higher gear after 1949 when John Enders, Thomas Weller and Frederick Robbins demonstrated the ability of poliomyelitis viruses to grow in cultures of various types of tissue. For making this fundamental discovery these three scientists were honoured with the Nobel Prize in Medicine in 1954. This technology provided a relatively easy and safe way to grow viruses in monolayer cell cultures and paved the way to a polio vaccine.

THE POLIO EXPERIENCE

In the 1950s and early 1960s there was intensive research to develop safe and effective polio vaccines. Jonas Salk focused on the development of a formaldehyde inactivated polio vaccine (IPV) with a virus grown in cell culture systems. The testing of the trivalent IPV began in 1952, results of the field trial were reported in 1955, and the vaccine was licensed in the USA in the same year. However, in 1955, during a rush to develop sufficient vaccine for widespread use, manufacturing failures resulted in inadequate formalin inactivation of the virus, causing many cases of active disease and death (a disaster now known as the 'Cutter incident'). As a result of this tragedy more rigorous safety testing for vaccines was implemented.

In parallel with the IPV development, Albert Sabin was working on a live, attenuated poliovirus vaccine (oral polio vaccine, OPV), which was licensed in the USA in 1963 and replaced IPV in many countries due to ease of oral administration, efficacy in inducing herd immunity and lower cost. Until the 1990s, OPV was the primary vaccine recommended in the USA and most of Europe. However, with the disappearance of polio in these and other regions, concerns about the rare occurrence of reversion to *virulence* and

Discovery of viruses as infectious agents

In 1884, the Chamberland–Pasteur filter was invented. It had pores smaller than bacteria, so it was possible to completely remove bacteria through the filter. In 1892, a new class of non-filterable infectious agents was discovered: the viruses. Due to their small size, viruses were not visible using conventional microscopes, and it was not until 1931, with the application of an electron microscope, that the first images of viruses were obtained.

In the early 20th century, the differences between viruses and bacteria began to emerge. The main obstacle encountered in studying viruses was the fact that they only multiply within living cells.

Figure 1.10 Child with polio. Polio has been eradicated in most countries of the world; however, outbreaks still occur in developing countries.

Karen Kasmauski/Science Faction/Getty Images.

release of live virulent vaccine-strain virus into the environment led to the reassessment of the OPV benefit–risk profile. This resulted in the introduction of a new high-potency IPV in many countries where polio has already been eliminated. In developing countries, however, OPV remains the first-choice vaccine due to its lower cost, high efficacy and ease of administration (Figure 1.10).

Polio immunisation opened the door to other live, attenuated virus vaccines, such as those against measles, mumps, rubella and varicella. In the 1970s, a combined measles-mumps-rubella (MMR) vaccine was developed to minimise the total number of injections in infants. Data from clinical trials with MMR demonstrated that a combination of *antigens* could be administered safely and effectively.

Despite many significant advances, the attenuation of pathogens was still based largely on empirical observations of virulence. A more targeted attenuation would not become possible until advances in molecular biology allowed *virulence determinants* to be specifically targeted for deletion or disruption.

WHO polio position paper

"Prior to polio eradication, national immunisation schedules should include either oral polio vaccine, inactivated polio vaccine, or a combination of both. Vaccine decisions should be based on assessments of the potential for importation of wild poliovirus (WPV) and subsequent transmission. High immunisation coverage is essential to ensure adequate population immunity. As long as WPV transmission has not been interrupted everywhere, all polio-free countries and areas remain at risk of re-importation, particularly from the remaining polio-endemic countries."

Source: WHO (2010)

FROM WHOLE CELLS TO SPLIT AND SUBUNIT VACCINES

Whole organism vaccines for pathogens, such as influenza or pertussis, presented barriers to acceptance due to their *reactogenicity* profile, eg up to 20% of vaccinees receiving the original form of whole inactivated influenza vaccine developed fever and malaise. The pertussis vaccine caused high rates of fever and was alleged to cause some cases of encephalitis in children. This was subsequently shown to be unsubstantiated, but there was a loss of public confidence and reduced vaccination coverage. These safety concerns prompted research on other approaches to the production of safer and more effective vaccines.

Vaccine technology in the late 20th century evolved from growing and producing pathogens on a large scale in cell culture to defining and selecting protective antigens. Antigen purification was historically initiated with the manufacture of split influenza vaccines,

The need for new technologies to develop new vaccines

When developing new vaccines, the most direct approach (which in general involves the whole pathogen) will be used unless there are overriding safety, immunogenicity or practical reasons that make this impossible. In such instances, alternative strategies are employed, such as purified, recombinant or conjugated antigens in conjunction with adjuvants, or the use of novel delivery systems.

whereby the influenza vaccine was treated with a solvent to dissolve or disrupt the viral lipid envelope. In the 1970s, the first split influenza vaccines were produced using these fragmentation and purification techniques.

Further progress in biotechnology has allowed components of pathogens to be isolated and produced in large quantities. This was an extension of the toxoid approach, which many years earlier showed that it was unnecessary to include the whole organism in some vaccines. By eliminating any unwanted pathogenic components like lipids and nucleoproteins, the high purity of these antigens resulted in vaccines with reduced reactogenicity and improved safety profiles. The subunit approach utilises selected and purified single proteins or antigens, such as pertussis proteins, which form the acellular vaccine, or pneumococcal polysaccharides. In general, split and subunit vaccines are less reactogenic compared with the whole pathogen but, in many instances, they also have reduced *immunogenicity*.

In the early 1980s, the recombinant protein concept, built on advances in genetic engineering from the 1970s onwards, enabled a further technological leap in vaccine development. In this technique, a section of DNA coding for an antigenic protein is inserted into an expression system and the protein encoded is produced in large quantities. The recombinant proteins are harvested and purified from the expression system for incorporation into the vaccine. The recombinant approach excels at achieving non-infectious, highly pure antigen; in addition, it allows the production of antigens in large quantities so providing more doses.

The DNA recombinant approach

The first hepatitis B virus (HBV) vaccine was developed in 1970 by a 3-fold inactivation of HBV in plasma from the blood of chronic HBV carriers (see *Chapter 3 — Vaccine antigens*). Particles of hepatitis B surface antigen found in their plasma were immunogenic and protective as a vaccine and did not cause infection. The first plasma-derived HBV vaccine was manufactured in 1981 with a very

good safety record, but despite extensive purification measures to inactivate any potential contaminating agents, physicians and the general public were very reluctant to use a product that carried even a remote theoretical risk of contamination with blood-borne agents. Moreover, as the vaccine depended on human serum from chronic carriers, sources of antigen were limited. These obstacles prompted the formulation of the first recombinant vaccine; an HBV vaccine that was as effective as the plasma-derived vaccine was licensed in 1986. This used the purified recombinant HBV surface antigen produced in a yeast expression system.

Since 2006, two additional recombinant vaccines have been made available. These prevent infection with human papillomavirus (HPV) types that cause cervical cancer (HPV-16, HPV-18), and one of the vaccines also prevents infection with HPV types causing genital warts (HPV-6, HPV-11). The vaccines consist of immunogenic virus-like particles (VLPs) prepared from recombinant HPV L1 coat protein in yeast, or insect cells. The coat proteins self-assemble during the purification process and mimic the overall structure of virus particles, but contain no HPV nucleic acid and cannot cause infection. However, as with all subunit vaccines and highly purified antigens obtained with peptide and recombinant technologies, HPV VLP vaccines require an adjuvant to enhance their ability to stimulate the immune system.

From polysaccharides to polysaccharide-conjugated vaccines

PNEUMOCOCCAL AND MENINGOCOCCAL VACCINES

A whole inactivated *Pneumococcus* vaccine was developed in 1911, long before the importance of type-specific immunity was known. It is now understood that the serotypic variations in *Pneumococci* make developing an effective vaccine extremely challenging (see *Chapter 2 — Vaccine immunology* and *Chapter 3 — Vaccine antigens*). In the late 1940s, a *multivalent* (4—6 types) capsular polysaccharide vaccine was developed; however, this was not used extensively as antibiotic therapy for pneumococcal infections became widely available at the same time.

During the 1970s and 1980s, several polyvalent bacterial vaccines consisting of purified capsular polysaccharides were developed as even though antibiotics were available, pneumococcal infections remained common and severe. Meningococcal polysaccharide group A and C vaccines were launched at the same time.

However, polysaccharide vaccines did not provide an adequate stimulus to the immature immune systems of children younger than 2 years of age, and older children and adults required revaccination every 3–5 years because of the limited duration of immunity. The preparation of vaccines by *conjugation* of polysaccharides to a protein carrier, typically tetanus or diphtheria toxoid, was introduced to overcome poor immunogenicity. The first 7-valent conjugated pneumococcus vaccine was developed in the 1990s followed in the 2000s by two formulations containing additional serotypes. Several group C meningococcal conjugates with either diphtheria or tetanus toxoid were developed in the 1990s. A-, C-, W- and Y-type polysaccharide-conjugated vaccines were then licensed in 2005. These provide a longer duration of immunity than the unconjugated polysaccharide vaccines, establish adequate *immune memory* and provide immune protection to those younger than 2 years of age.

HAEMOPHILUS INFLUENZA VACCINES

In 1892, *Haemophilus influenzae* type b (Hib), the most common cause of invasive bacterial disease, was isolated. In the 1930s, the role of the Hib polysaccharide capsule as a *virulence factor* in the disease was identified. The first attempts to develop an Hib vaccine started in the 1970s and a vaccine was licensed in 1985. As with other polysaccharide vaccines, this vaccine had limited immunogenicity and was not effective in children younger than 18 months. The first conjugated Hib vaccine, licensed in 1987, had excellent efficacy and immunogenicity, even in infants. Several Hib vaccines were licensed in the early 1990s and their widespread use has eliminated much of the Hib disease in Western countries.

The improved pneumococcal, meningococcal and Hib-conjugated vaccines have led to massive reductions in cases of invasive disease in children and adolescents, and reductions in the overall number of cases in the whole population due to the benefits of herd immunity.

From live attenuated to reassortant viruses

New vaccine technologies appeared in the 1990s, including *reassortment* and cold adaptation, which made it possible to develop successful live, attenuated influenza vaccines. Understanding of the molecular mechanisms involved in viral

TABLE 1.2. IMPACT OF VACCINES IN THE USA

	Pre-vaccination – estimated annual average		Post-vaccination (year)	
Disease	**Cases**	**Deaths**	**Cases**	**Deaths**
Diphtheria	21,053	1822	0 (2006)	0 (2004)
Measles	530,217	440	55 (2006)	0 (2004)
Mumps	162,344	39	6584 (2006)	0 (2004)
Pertussis	200,752	4034	15,632 (2006)	27 (2004)
Poliomyelitis, acute	19,794	1393	0 (2006)	0 (2004)
Poliomyelitis, paralytic	16,316	1879	0 (2006)	0 (2004)
Rubella	47,745	17	11 (2006)	0 (2004)
Congenital rubella syndrome	152	Not available	1 (2006)	0 (2004)
Smallpox	29,005	337	0 (2006)	0 (2004)
Tetanus	580	472	41 (2006)	4 (2004)
Hepatitis A	117,333	137	3579 (2006)	18 (2006)
Acute hepatitis B	66,232	237	4713 (2006)	47 (2006)
Invasive Hib	20,000	1000	208 (2006)	<5 (2006)
Invasive pneumococcal disease	63,067	6500	5169 (2006)	4850 (2006)
Varicella	4,085,120	105	48,445 (2006)	19 (2006)

Adapted from Roush and Murphy (2007).
Hib, Haemophilus influenzae type b.

Adherence to vaccination programmes is of the utmost importance for the control or eradication of infectious diseases

There are several examples, such as the outbreak of pertussis in Japan in 1975 and of measles in the UK in 2006, showing how diseases once close to eradication in particular regions can re-emerge because vaccination coverage declines below a critical threshold.

Following initiation of widespread vaccination of children in the late 1950s, diphtheria was well-controlled and outbreaks were uncommon in the Soviet Union for more than two decades. After the break-up of the Soviet Union, there was a collapse of the public health infrastructure including vaccination programmes. In 1990, a massive diphtheria epidemic was observed in the successor states, resulting in more than 4000 deaths (CDC, 1996).

In Nigeria in the 1990s, a rumour that the polio vaccine caused sterility resulted in large portions of the population refusing to be vaccinated. This misinformation and vaccination breakdown resulted in the 2009 polio outbreak in Nigeria and polio is currently spreading to neighbouring countries. Similarly, Tajikistan, which had been polio-free since 1996, was reinfected with poliovirus from northern India in 2010. By mid-May 2010, paralysis was reported in more than 430 children (WHO, 2010). The WHO notes that events such as these indicate a threat to the goal of a polio-free world.

attenuation led to the development of *reassortant* technology (see *Chapter 3 — Vaccine antigens*). Co-infection of cell culture with wild and attenuated strains allows the viruses to 'swap' genome segments, producing new variants with desirable genetic components selectively derived from multiple strains. This technique is possible in viruses, such as the rotavirus, where the genome of the organism is arranged in physically separate RNA segments. Co-infection of cell cultures with different strains results in viruses containing genetic material from all strains. A pentavalent rotavirus vaccine licensed in 2006 is based on an attenuated bovine rotavirus reassorted with human rotavirus segments.

Conclusion

Vaccination programmes have helped to significantly reduce the number of reported cases of diseases worldwide (Table 1.2 summarises the impact of vaccines in the USA). Successful eradication of diseases can be achieved through vaccination of pathogens that have no human reservoir, are non-variable and have solid immunity/no *latency*. Smallpox is the first success story and eradication of polio is a distinct possibility having already been eradicated from many regions of the world. For many other diseases that meet these criteria, eradication is on the horizon with the ongoing development of many new vaccines (see *Chapter 6 — Vaccines of the future*).

The majority of vaccines being developed today use technologies based on a better understanding of immune responses, the ability to generate the antigen on a mass scale and our increased knowledge of host—pathogen interactions. At present, the focus is on subunit (purified protein or polysaccharide), genetically engineered and vectored antigens (see *Chapter 3 — Vaccine antigens*). Most recently, the key role played by *antigen-presenting cells* in the connection between the innate and *adaptive immune systems* has been recognised. The discovery of the immunological interplay between immune cells of these systems has opened new doors in vaccine design (see *Chapter 2 — Vaccine immunology*).

Knowledge of how pathogens evoke the defensive triggers of the immune system, together with a better understanding of how immune cells subsequently react and develop an immune response, has prompted much research in improving the visibility of the antigen to the *innate immune system*. Among other areas of ongoing research (see *Chapter 6 — Vaccines of the future*), the use of adjuvants is seen today as one of the most promising and advanced approaches in guiding the immune system to an appropriate immune response to the vaccine antigen (see *Chapter 4 — Vaccine adjuvants*).

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